International Journal of Cardiology 179 (2015) 58–60

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Plasma cancer biomarker multiplex screening and the risk of subsequent preeclampsia

Margarita L. Martinez-Fierro [a,*], Idalia Garza-Veloz [a], Claudia Castruita-Dela Rosa [a], Yolanda Ortiz-Castro [a], Maria C. Aceves-Medina [b], Rosbel Vazquez-Castro [c], Ivan Delgado-Enciso [d,e], Maria E. Castañeda-Lopez [a]

[a] Molecular Medicine Laboratory, Unidad Academica de Medicina Humana y Ciencias de la Salud, Carretera Zacatecas-Guadalajara Km.6, Ejido la Escondida, Zacatecas, Mexico
[b] Biomedical Research Laboratory, Unidad Academica de Odontologia, Carretera Zacatecas-Guadalajara Km.6, Ejido la Escondida, Zacatecas, Mexico
[c] Hospital de la Mujer Zacatecana, Servicios de Salud de Zacatecas, Av. Programacion y Presupuesto 7, Zona Industrial, Zacatecas, Mexico
[d] School of Medicine, University of Colima, Av. Universidad 333, Colonia las viboras, Colima, Mexico
[e] Instituto Estatal de Cancerologia, Servicios de Salud del Estado de Colima, Mexico

ARTICLE INFO

Article history:
Received 21 October 2014
Accepted 27 October 2014
Available online 30 October 2014

Keywords:
Preeclampsia
Plasma biomarkers
Cancer protein screening
Angiogenesis
Placenta

Preeclampsia (PE) is a pregnancy multisystem disorder that is characterized by hypertension and proteinuria that develops after the 20th gestational week (GW). The placenta abnormal implantation process is well known to play a major role in the development of disease [1]. The molecular mechanisms that are involved in normal placental implantation are tightly related to the proliferative and invasive capacity of trophoblast cells and recent evidence suggests a common convergence between PE genes and those involved in tumor progression [2–4]. Although the invasion process is increased in cancer and occurs to a lesser extent in PE, known cancer genes involved in tumor progression may be useful as potential PE biomarkers and could play a role in the PE etiopathogenesis. Because to date, no biomarkers have been successfully validated for accurate identification of patients at risk for the PE development, in this study, we evaluate the usefulness of predicting PE based on the composite profiling of 34 plasma cancer-related proteins.

We performed a nested cohort case–control study in which the participants were drawn from a cohort of 588 pregnant women followed from the first trimester to delivery in the Centro de Salud Urbano "José Castro Villagrana" and the Hospital de la Mujer Zacatecana, in Zacatecas, Mexico, between November 2011 and January 2014. The criteria for PE diagnosis and its severity/onset sub-classifications were established according as described in previous investigations [1,5]. The study exclusion criteria included gestational hypertension, gestational diabetes mellitus, and underlying medical diseases. All cohort patients provided written informed consent for their participation, and the Institutional Review Board approvals were obtained (ID: ACS/UAZ.Ofc.No.0042010-06/2014 and HMZ-117/14). The participants provided peripheral blood starting on the 12th, 16th and/or 20th GW. Patients who had a PE diagnosis during the follow-up provided a fourth blood sample at the time of diagnosis. Sixteen women who developed PE during the follow-up period were selected and individually matched based on maternal age, null-parity, personal/family histories of PE, and body mass index (BMI), to 18 women who had healthy pregnancies without complications. A total of 91 plasma samples were obtained, with 22 corresponding to samples donated on 12 GW (6 from women predicted to develop PE (WPD-PE) and 16 from controls), 23 on 16 GW (8 from WPD-PE and 15 from controls), 31 on 20 GW (15 from WPD-PE and 16 from controls), and with the remaining 15 being from the WPD-PE at the time of the PE diagnosis. Cell-free plasma was obtained from blood samples collected in tubes with EDTA as described previously [6] and stored at −80 °C until assayed. The plasma measurements of 34 proteins were assayed in duplicate using the Bio-Plex Pro™ Human Cancer Biomarker Panel 1, and 2 (171-AC500M and 171-AC600M: Bio-Rad, Hercules, CA, USA) kits, according to the manufacturer's instructions using 200-μl of sample. The final reaction mixtures were quantified using the Bio-Plex MAGPIX® instrument and the data were processed and analyzed using Bio-Plex Manager Software 6.1 (all Bio-Rad). Evaluation of simple biomarker comparisons between the WPD-PE and the controls, and/or according to PE severity or onset of disease, was performed using Student's t-test or Mann–Whitney rank sum test.

Abbreviations: PE, preeclampsia; FGF-basic, fibroblast growth factor 2; sHER-2/neu, soluble v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; sIL-6Rα, soluble interleukin 6 receptor alpha; IGFBP-1, insulin-like growth factor binding protein 1; uPA, plasminogen activator urokinase; GW, gestation weeks; BMI, body mass index; ROC, receiver operating characteristic curve; OR, odds ratio; WPD-PE, women predicted to develop preeclampsia; PPV, predictive positive value; PNV, predictive negative value.

* Corresponding author at: Unidad Academica de Medicina Humana y Ciencias de la Salud, Universidad Autonoma de Zacatecas, Carretera Zacatecas-Guadalajara Km.6, Ejido la Escondida, CP 98160 Zacatecas, Zacatecas, Mexico.
E-mail address: margaritamf@uaz.edu.mx (M.L. Martinez-Fierro).

http://dx.doi.org/10.1016/j.ijcard.2014.10.168

Concentration differences among the time points were evaluated using Kruskal–Wallis one way analysis of variance on ranks coupled to Dunn's method as a multiple comparison procedure. The PE predictive capacity of proposed proteins was evaluated using a Receiver Operating Characteristic curve (ROC) analysis. The odds ratios (OR) with Yates continuity correction were calculated for significant comparisons. Statistical analysis was performed considering a significance level of 0.05 with the software Sigma Plot v.11 (Systat Software Inc., San Jose, CA) and GraphPad Prism v.5.03 (Graph-Pad Software, San Diego, California, USA).

The 25% of the cases were diagnosed with early PE, and 31% were subclassified as severe PE. Fig. 1 shows that, based on the 34 markers analyzed, three of them at 16 GW (FGF-basic, sHER2/neu, and uPA) and four at 20 GW (sHER2/neu, sIL-6α, endoglin, and IGFBP-1) had differences between the WPD-PE and controls (P values < 0.05). Differences in the plasma concentration of these markers between the severe and mild PE patients nor between the patients with early and late disease onset were not observed (P values > 0.05; data not shown). The evaluation of the potential usefulness of the markers identified to classify pregnant women by their risk of developing PE (Table 1) showed that, on an individual basis, FGF-basic and uPA showed a sensitivity of 100% at 16 GW whereas sIL-6Rα showed a sensitivity of 93% at 20 GW. The best specificity value (100%) was observed for sHER2/neu at 16 GW. Although the individual markers identified with high sensitivity values had low specificity (in the range of 50–69%) and vice-versa, the concentration cutoff values obtained allowed us to calculate up to 18-fold increased risk for PE development among the study population. The classificatory capacity for PE development increased when FGF-basic and uPA were used together; their ratio allowed us to classify WPD-PE as early as 16 GW with the lowest values of false negative (0.0%) and false positive (27%) rates and therefore with higher sensitivity and specificity values (100% and 79%, respectively). There are no previous studies that have evaluated the PE predictive capacity of those proteins together in maternal plasma during the first and second trimesters of pregnancy and only a few longitudinal reports have evaluated molecules with PE predictive capacity [7–10]. Despite one of the developed algorithms for the calculation of patient-specific risk, had acceptable rates of false-positive (5%), it involves a large combination of variables (maternal/clinical factors, serum biomarkers and ultrasound measurements) [7] that make the risk determination difficult. This study proposes a new set of biomarkers for PE prediction and suggests that

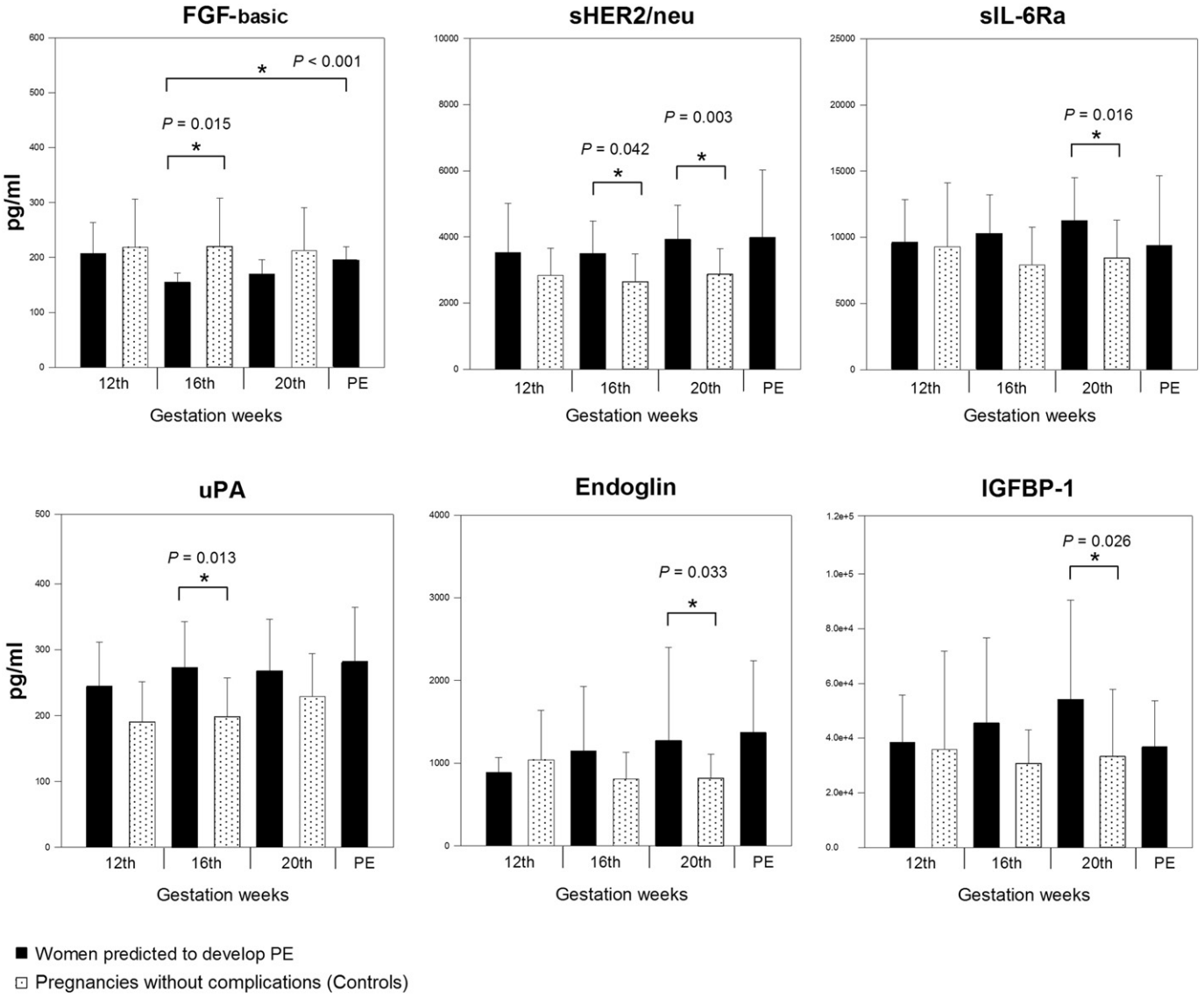

Fig. 1. Concentration data of the significant markers at the four pregnancy time points. Pregnancies were classified as WPD-PE or as pregnancies without complications, and the concentrations (pg/ml) of each marker were compared between the groups at each time point. The figure shows the results obtained for FGF-basic, sHER2/neu, sIL-6α, uPA, endoglin, and IGFBP-1. For the WPD-PE, a set of 15 samples at the time of the PE diagnosis was included in the plot; this sample set was not available for the controls.

Table 1
ROC analysis of the significant markers.

Parameter	16 GW				20 GW			
	FGFb	sHER2/neu	uPA	FGFb/uPA	sHER2/neu	sIL-6Ra	Endoglin	IGFBP-1
Area under ROC curve	0.82	0.76	0.77	0.86	0.83	0.78	0.73	0.74
Cutoff (pg/ml)	187.4	3798	207.9	0.75	3757	8255	869	34,327
Sensitivity	1	0.500	1	1	0.67	0.93	0.73	0.8
Specificity	0.67	1	0.67	0.79	0.88	0.56	0.69	0.69
PPV	0.62	1	0.62	0.73	0.83	0.67	0.69	0.69
PNV	1	0.79	1	1	0.74	0.90	0.73	0.73
Odds ratio	ND	ND	ND	ND	14.00	18.00	6.10	6.10
95% CI	ND	ND	ND	ND	2.3–87.2	1.9–171.9	1.3–28.7	1.3–28.7
P value	0.003*	0.008*	0.003*	0.001*	0.006*	0.006*	0.047*	0.047*

PPV: predictive positive value; PNV: predictive negative value; ROC: receiver operating characteristic; ND: not determined.

FGF-basic, sHER2/neu sIL-6Rα, endoglin, and IGFBP-1 may be involved in the pathogenesis of disease. Future longitudinal studies will be necessary to validate the markers proposed as an early PE screening test.

Acknowledgments

Authors thank all the study participants. We are grateful to the clinical staff of the Centro de Salud "José Castro Villagrana" and from the Hospital de la Mujer Zacatecana for their invaluable assistance in obtaining samples. We also gratefully acknowledge the support in the patient recruitment by Dr. Raúl Hernández León (SSZ), and AdrianaVega-Belmont, and Cecilia Adan-Meza, for her support in the data collection. This work was supported by the National Council for Science and Technology (CONACyT), Mexico [CONACYT-SEP-CB-128567, CONACYT-Gobierno del Estado de Zacatecas-203220, CONACyT-SSA/IMSS/ISSSTE-138721,-181124, and CONACyT-INFR-225520 to M.L.M.F.].

References

[1] I. Garza-Veloz, C. Castruita-De la Rosa, R. Cortes-Flores, V. Martinez-Gaytan, J.E. Rivera-Munoz, E.A. Garcia-Mayorga, et al., No association between polymorphisms/haplotypes of the vascular endothelial growth factor gene and preeclampsia, BMC Pregnancy Childbirth 11 (2011) 35.

[2] F. Louwen, C. Muschol-Steinmetz, J. Reinhard, A. Reitter, J. Yuan, A lesson for cancer research: placental microarray gene analysis in preeclampsia, Oncotarget 3 (2012) 759–773.

[3] R. Moslehi, J.L. Mills, C. Signore, A. Kumar, X. Ambroggio, A. Dzutsev, Integrative transcriptome analysis reveals dysregulation of canonical cancer molecular pathways in placenta leading to preeclampsia, Sci. Rep. 3 (2013) 2407.

[4] C. Ferretti, L. Bruni, V. Dangles-Marie, A.P. Pecking, D. Bellet, Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts, Hum. Reprod. Update 13 (2007) 121–141.

[5] M.A. Brown, M.D. Lindheimer, M. de Swiet, A. Van Assche, J.M. Moutquin, The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP), Hypertens. Pregnancy 20 (2001) IX–XIV.

[6] S.S. Chim, T.K. Shing, E.C. Hung, T.Y. Leung, T.K. Lau, R.W. Chiu, et al., Detection and characterization of placental microRNAs in maternal plasma, Clin. Chem. 54 (2008) 482–490.

[7] L.C. Poon, N.A. Kametas, N. Maiz, R. Akolekar, K.H. Nicolaides, First-trimester prediction of hypertensive disorders in pregnancy, Hypertension 53 (2009) 812–818.

[8] A.O. Odibo, Y. Zhong, K.R. Goetzinger, L. Odibo, J.L. Bick, C.R. Bower, et al., First-trimester placental protein 13, PAPP-A, uterine artery Doppler and maternal characteristics in the prediction of pre-eclampsia, Placenta 32 (2011) 598–602.

[9] D. Chen, M. Dong, Q. Fang, J. He, Z. Wang, X. Yang, Alterations of serum resistin in normal pregnancy and pre-eclampsia, Clin. Sci. (Lond.) 108 (2005) 81–84.

[10] S. Muttukrishna, R.A. North, J. Morris, J.C. Schellenberg, R.S. Taylor, J. Asselin, et al., Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia, Hum. Reprod. 15 (2000) 1640–1645.